Invited Commentary:

Calcineurin inhibitor nephrotoxicity in the era of antibody-mediated rejection

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Commentary

Brian Nankivell and the transplant team of Westmead Hospital in Sydney, who published a seminal paper on the evolution of kidney allograft histology more than a decade ago (1), today present a new addition to their work. In this issue of *Transplantation*, they echo their conclusion of 2003, that the calcineurin inhibitors (CNIs) cyclosporine and tacrolimus are imperfect maintenance immunosuppressive agents. “One kidney for life will remain largely unrealized with CNI dependent therapy” (2).

Acute and chronic CNI nephrotoxicity, both from cyclosporine and tacrolimus, was already recognized in the first human experience with cyclosporine (3-5). Because the introduction of these powerful immunosuppressants dramatically decreased the risk of acute rejection and of rejection-associated graft failure, the nephrotoxicity of cyclosporine was accepted by the transplant community, and considered as collateral damage.

In 2003, Nankivell and colleagues described the kinetics and long-term evolution of protocol biopsy histology in combined kidney-pancreas transplant recipients (1), of patients transplanted between 1987 and 2000, a transplant era not comparable to current clinical practice. Strikingly, histology of chronic CNI nephrotoxicity, defined as striped cortical fibrosis or new-onset arteriolar hyalinosis, and supported by tubular microcalcifications, was virtually universal at 10 years after transplantation.

This last finding, taken together with the analysis that long-term graft outcome had not improved between 1988 and 1995 (6), strongly supported the common thinking of the 2000s, that CNI sparing is the way to move forward and to attain long-term outcome improvement. The largest studies in the field of kidney transplantation were performed with this primary goal (7), and are ongoing as of today (e.g. the TRANSFORM study; clinicaltrials.gov NCT01950819). These studies attempt to minimize, or completely avoid, exposure to CNIs, while maintaining effective rejection prevention.

Despite the extensive literature irrefutably confirming the very existence of CNI nephrotoxicity (5), the numerous trials in this field were largely disappointing. CNI-sparing regimens lead to improved kidney transplant function, but also to an increased risk of acute rejection (8). CNI-minimizing regimens (maintaining CNIs but a lower dose) showed a reduction in graft failure, while avoidance studies, which substituted CNIs by mTOR inhibitors, showed an increase in graft failure (9). These meta-analyses considered the class effects of CNIs, without accounting for potential differences between cyclosporine- and tacrolimus-based regimens, e.g. absence of graft functional improvement when switching from tacrolimus to an mTOR inhibitor (10). Taken together, these analyses support current routine clinical practice in most transplant centers, to use low-dose CNIs as mainstay of the immunosuppressive regimen after kidney transplantation. Calcineurin inhibitors: can't live with, can't live without.

While the jury is still out on the position of CNI-sparing immunosuppressive regimens for kidney transplantation, much of the interest of the transplant community has shifted towards antibody-mediated rejection. Being neglected for many decades, the advent of highly sensitive and specific methods to evaluate the presence of circulating donor-specific HLA antibodies (11), better insight in the specific histology of antibody-mediated rejection (12), and publications illustrating the prime role of antibody-mediated rejection in kidney graft failure (13-15), sparked interest in its risk factors, phenotype and therapies.

All these evolutions culminate in the new single-center analysis by Brian Nankivell and his team, published today in *Transplantation* (2). In a cohort analysis on 200 recipients of combined kidney-pancreas grafts, with a unique long-term follow up with protocol biopsies up to 10 years after transplantation, the histological evolution of the kidney grafts was compared between the cyclosporine era (1988-1999; most often with concomitant azathioprine) and the tacrolimus era (1999-2012; concomitant mycophenolate mofetil). Given the very different context, the comparison between cyclosporine and tacrolimus is unfair. This study should not be read as a direct comparison

between both CNIs, but as a comparison between very different transplant eras. This is not a randomized trial, and we should not misinterpret it as one.

Evaluating protocol biopsy histology, what changed over the past two decades? First, and perhaps most importantly, pretransplant sensitization with donor-specific HLA antibodies has decreased importantly, from 44% to 18% of patients (2). Although not specified by the authors, this is likely due to the fact that in the earliest time frame, currently used sensitive techniques were not available, and patients with CDC negative crossmatch were transplanted while lower grade donor-specific antibodies were missed. This translated in a dramatic drop in early antibody-mediated rejection from 32% to 6%, and also acute cellular rejection and vascular rejection were significantly less frequent in the tacrolimus compared to the cyclosporine era.

Furthermore, the previously described association between tubulo-interstitial inflammation and progressive fibrosis (16), and the lower incidence of inflammation in the more recent era in the new study by Nankivell and his team (2) could partly explain the finding that interstitial fibrosis progressed more slowly in the more recent tacrolimus era. However, it is acknowledged that causal relations cannot be evaluated in this cohort study, and the difference in fibrosis was no longer significant by 10 years after transplantation (2). This illustrates that also other factors could play a role in progressive fibrosis. Moreover, recent data from the belatacept studies illustrate that rejection, fibrosis and outcome are not inseparably related to each other. Interstitial fibrosis, graft function and long-term graft outcome were best in the belatacept-treated study arms, while these study arms had the highest rejection rates (17, 18). Taken together, it is time to reemphasize the role of tubulo-interstitial inflammation or rejection in the progression of fibrosis.

The important background differences between the two populations studied (2) obviates drawing firm conclusions from the comparisons. Nevertheless, a conclusion that can be drawn from these data is that a main aspect of CNI nephrotoxicity, arteriolar hyalinosis, was not remarkably better in recent tacrolimus-treated patients than in cyclosporine-treated patients. Also subsequent

glomerulosclerosis, likely associated with vascular luminal narrowing, decreased blood flow and altered glomerular hemodynamics, was similar in the tacrolimus era compared to the cyclosporine era.

Moreover, in detailed supplementary analyses, Nankivell et al illustrate that the relation between CNI exposure and progressive histological injury is poor, questioning the clinical relevance of maintaining low peripheral blood CNI levels (CNI minimization) to avoid CNI nephrotoxicity (2). Minimizing or avoiding CNI exposure could well be risky in terms of donor-specific antibody formation (19, 20), with only limited benefits on CNI nephrotoxicity.

Therefore, the real question that arises is not whether CNI nephrotoxicity exists, but what are its consequences. Does the collateral damage of chronic CNI use exceed the immunosuppressive effects?

Despite the progressive nature of CNI nephrotoxicity, it remains very unclear whether this progressive histological injury translates in impaired graft function or decreased graft failure on the long-term. Moreover, it is necessary to remind us that two earlier studies demonstrated an association between presence of arteriolar hyalinosis and better graft outcome (15, 21). This is counterintuitive but could be related to the powerful immunosuppressive effects of CNIs, perhaps surpassing their nephrotoxic effects.

Also in this new study (2), better graft survival at one year after transplantation in the tacrolimus era compared to the cyclosporine era (96% vs. 89%), despite comparable arteriolar hyalinosis, perhaps supports the suggestion that it is not arteriolar hyalinosis in itself that explains graft failure. Long-term follow-up of these patients, and evaluation of the association between histology of CNI nephrotoxicity, subsequent glomerulosclerosis, and graft outcome, should shed additional light on this intriguing issue.

In conclusion, biopsies performed late after transplantation usually present with a complex histological picture. Albert Einstein once said: “Everything should be as simple as it can be, but not

simpler". Also in the field of kidney allograft histology, we should not oversimplify the histological phenotypes. It is time to accept the complexity of the histological phenotypes after kidney transplantation, and recognize that allo-immune phenomena co-exist with other phenotypes like CNI nephrotoxicity.

We need to embrace this complexity, and continue our search for effective prevention of kidney allograft rejection, while avoiding the long-term use of nephrotoxic agents.

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